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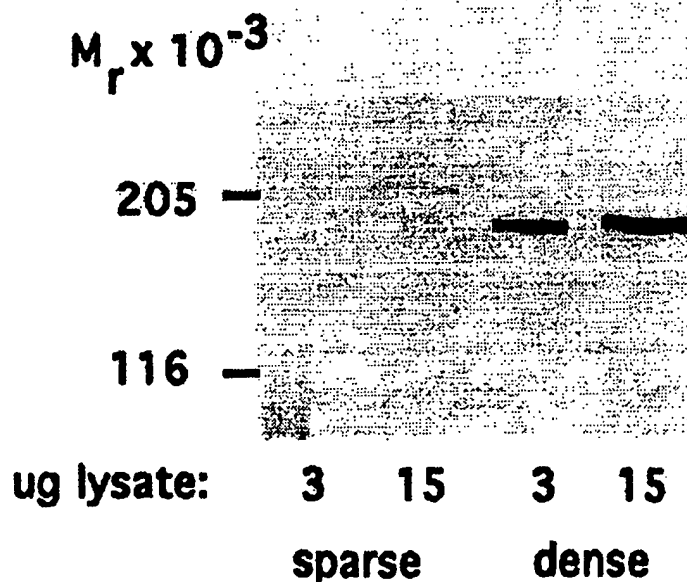
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(54) Title: DENSITY ENHANCED PROTEIN TYROSINE PHOSPHATASES

(57) Abstract

Novel Type III density enhanced protein tyrosine phosphatases are disclosed and exemplified by human DEP-1 enzyme. Polynucleotides encoding huDEP-1 are disclosed, along with methods and materials for production of the same by recombinant procedures. Binding molecules specific for DEP-1 are also disclosed as useful for modulating the biological activities of DEP-1.



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DENSITY ENHANCED PROTEIN TYROSINE PHOSPHATASES

FIELD OF THE INVENTION

The present invention relates generally to purified and isolated protein tyrosine phosphatase enzymes (PTPs) and polynucleotides encoding the same. PTPs of the invention are characterized by upregulated mRNA transcription and/or translation, or post-translational modification leading to increased total cellular enzyme activity as a function of increased cellular contact with neighboring cells. Such density enhanced PTPs are referred to as DEPTPs. An illustrative human Type III receptor-like density-enhanced protein tyrosine phosphatase has been designated huDEP-1.

BACKGROUND OF THE INVENTION

Protein tyrosine phosphorylation is an essential element in signal transduction pathways which control fundamental cellular processes including growth and differentiation, cell cycle progression, and cytoskeletal function. Briefly, the binding of growth factors, or other ligands, to a cognate receptor protein tyrosine kinase (PTK) triggers autophosphorylation of tyrosine residues in the receptor itself and phosphorylation of tyrosine residues in the enzyme's target substrates. Within the cell, tyrosine phosphorylation is a reversible process; the phosphorylation state of a particular tyrosine residue in a target substrate is governed by the coordinated action of both PTKs, catalyzing phosphorylation, and protein tyrosine phosphatases (PTPs), catalyzing dephosphorylation.

The PTPs are a large and diverse family of enzymes found ubiquitously in eukaryotes [Charbonneau and Tonks, *Ann.Rev.Cell Biol.* 8:463-493 (1993)]. Structural diversity within the PTP family arises primarily from variation in non-catalytic (potentially regulatory) sequences which are linked to one or more highly conserved catalytic domains. In general, soluble cytoplasmic PTP forms are termed non-receptor PTPs and those with at least one non-catalytic region that traverses the cell membrane are termed receptor-like PTPs (RPTPs).

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A variety of non-receptor PTPs have been identified which characteristically possess a single catalytic domain flanked by non-catalytic sequences. Such non-catalytic sequences have been shown to include, among others, sequences homologous to cytoskeletal-associated proteins [Yang and Tonks, *Proc.Natl.Acad.Sci.(USA)* 88:5949-5953 (1991)] or to lipid binding proteins [Gu, *et al.*, *Proc.Natl.Acad.Sci.(USA)* 89:2980-2984 (1992)], and/or sequences that mediate association of the enzyme with specific intracellular membranes [Frangioni *et al.*, *Cell* 68:545-560 (1992)], suggesting that subcellular localization may play a significant role in regulation of PTP activity.

Analysis of non-catalytic domain sequences of RPTPs suggests their involvement in signal transduction mechanisms. However, binding of specific ligands to the extracellular segment of RPTPs has been characterized in only a few instances. For example, homophilic binding has been demonstrated between molecules of PTP μ [Brady-Kalnay, *et al.*, *J.Cell. Biol.* 122:961-972 (1993)] *i.e.*, the ligand for PTP μ expressed on a cell surface is another PTP μ molecule on the surface of an adjacent cell. Little is otherwise known about ligands which specifically bind to, and modulate the activity of, the majority of RPTPs.

Many receptor-like PTPs comprise an intracellular carboxyl segment with two catalytic domains, a single transmembrane domain and an extracellular amino terminal segment [Krueger *et al.*, *EMBO J.* 9:3241-3252 (1990)]. Subclasses of RPTPs are distinguished from one another on the basis of categories or "types" of extracellular domains [Fischer, *et al.*, *Science* 253:401-406 (1991)]. Type I RPTPs have a large extracellular domain with multiple glycosylation sites and a conserved cysteine-rich region. CD45 is a typical Type I RPTP. The Type II RPTPs contain at least one amino terminal immunoglobulin (Ig)-like domain adjacent to multiple tandem fibronectin type III (FNIII)-like repeats. Similar repeated FNIII domains, believed to participate in protein:protein interactions, have been identified in receptors for IL2, IL4, IL6, GM-CSF, prolactin, erythropoietin and growth hormone [Patthy, *Cell* 61:13-14 (1992)].

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The leukocyte common antigen-related PTP known as LAR exemplifies the Type II RPTP structure [Streuli *et al.*, *J.Exp.Med.* 168:1523-1530 (1988)], and, like other Type II RPTPs, contains an extracellular region reminiscent of the NCAM class of cellular adhesion molecules [Edelman and Crossin, *Ann.Rev.Biochem.* 60:155-190 (1991)]. The Type III RPTPs, such as HPTP β [Krueger *et al.*, *EMBO J.* 9:3241-3252 (1990)], contain only multiple tandem FNIII repeats in the extracellular domain. The Type IV RPTPs, for example RPTP α [Krueger *et al.* (1990) *supra*], have relatively short extracellular sequences lacking cysteine residues but containing multiple glycosylation sites. A fifth type of RPTP, exemplified by PTP γ [Barnes, *et al.*, *Mol.Cell.Biol.* 13:1497-1506 (1993)] and PTP ζ [Krueger and Saito, *Proc.Natl.Acad.Sci.(USA)* 89:7417-7421 (1992)], is characterized by an extracellular domain containing a 280 amino acid segment which is homologous to carbonic anhydrase (CAH) but lacks essential histidine residues required for reversible hydration of carbon dioxide.

FNIII sequences characteristically found in the extracellular domains of Type II and Type III RPTPs comprise approximately ninety amino acid residues with a folding pattern similar to that observed for Ig-like domains [Bork and Doolittle, *Proc.Natl.Acad.Sci(USA)* 89:8990-8994 (1992)]. Highly conserved FNIII sequences have been identified in more than fifty different eukaryotic and prokaryotic proteins [Bork and Doolittle, *Proc.Natl.Acad.Sci.(USA)* 89:8990-8994 (1992)], but no generalized function has been established for these domains. Fibronectin itself contains fifteen to seventeen FNIII domain sequences, and it has been demonstrated that the second FNIII domain (FNIII₂) contains a binding site for heparin sulphate proteoglycan [Schwarzbauer, *Curr.Opin.Cell Biol.* 3:786-791 (1991)] and that FNIII₁₃ and FNIII₁₄ are responsible for heparin binding through ionic interactions [Schwarzbauer, *Curr.Opin.Cell Biol.* 3:786-791 (1991)]. Perhaps the best characterized interaction for a fibronectin FNIII domain involves FNIII₁₀ which is the major site for cell adhesion [Edelman and Crossin, *Ann.Rev.Biochem*

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60:155-190 (1991); Leahy, *et al.*, *Science* 258:987-991 (1992); Main, *et al.*, *Cell* 71:671-678 (1992)]. FNIII₁₀ contains the amino acid sequence Arg-Gly-Asp (RGD) which is involved in promoting cellular adhesion through binding to particular members of the integrin superfamily of proteins.

5 Characteristics shared by both the soluble PTPs and the RPTPs include an absolute specificity for phosphotyrosine residues, a high affinity for substrate proteins, and a specific activity which is one to three orders of magnitude in excess of that of the PTKs *in vitro* [Fischer, *et al.*, *Science* 253:401-406 (1991); Tonks, *Curr.Opin.Cell.Biol.* 2:1114-1124 (1990)]. This latter
10 characteristic suggests that PTP activity may exert an antagonistic influence on the action of PTKs *in vivo*, the balance between these two thus determining the level of intracellular tyrosine phosphorylation. Supporting a dominant physiological role for PTP activity is the observation that treatment of NRK-1 cells with vanadate, a potent inhibitor of PTP activity, resulted in enhanced levels of
15 phosphotyrosine and generation of a transformed cellular morphology [Klarlund, *Cell* 41:707-717 (1985)]. This observation implies potential therapeutic value for PTPs and agents which modulate PTP activity as indirect modifiers of PTK activity, and thus, levels of cellular phosphotyrosine.

 Recent studies have highlighted aspects of the physiological
20 importance of PTP activity. For example, mutations in the gene encoding a non-receptor hematopoietic cell protein tyrosine phosphatase, HCP, have been shown to result in severe immune dysfunction characteristic of the *motheaten* phenotype in mice [Schultz, *et al.*, *Cell* 73:1445-1454 (1993)]. Under normal conditions HCP may act as a suppressor of PTK-induced signaling pathways, for example,
25 the CSF-1 receptor [Schultz, *et al.*, *Cell* 73:1445-1454 (1993)]. Some PTP enzymes may be the products of tumor suppressor genes and their mutation or deletion may contribute to the elevation in cellular phosphotyrosine associated with certain neoplasias [Brown-Shimer, *et al.*, *Cancer Res.* 52:478-482 (1992); Wary, *et al.*, *Cancer Res.* 53:1498-1502 (1993)]. Mutations observed in the gene

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for RPTP γ in murine L cells would be consistent with this hypothesis [Wary, *et al.*, *Cancer Res.* 53:1498-1502 (1993)]. The observation that the receptor-like PTP CD45 is required for normal T cell receptor-induced signalling [Pingel and Thomas, *Cell* 58:1055-1065 (1989)] provides evidence implicating PTP activity
5 as a positive mediator of cellular signalling responses.

Normal cells in culture exhibit contact inhibition of growth, *i.e.*, as adjacent cells in a confluent monolayer touch each other, their growth is inhibited [Stoker and Rubin, *Nature* 215:171-172 (1967)]. Since PTKs promote cell growth, PTP action may underlie mechanisms of growth inhibition. In Swiss
10 mouse 3T3 cells, a phosphatase activity associated with membrane fractions is enhanced eight-fold in confluent cells harvested at high density as compared to cells harvested from low or medium density cultures [Pallen and Tong, *Proc.Natl.Acad.Sci.(USA)* 88:6996-7000 (1991)]. This elevated activity was not observed in subconfluent cell cultures brought to quiescence by serum deprivation.
15 The enhanced phosphatase activity was attributed to a 37 kD protein, as determined by gel filtration, but was not otherwise characterized. Similarly, PTPs have been directly linked to density arrest of cell growth; treatment of NRK-1 cells with vanadate was able to overcome density dependent growth inhibition and stimulate anchorage independent proliferation, a characteristic unique to
20 transformed, or immortalized, cells [Klarland, *Cell* 41:707-717 (1985); Rijksen, *et al.*, *J.Cell Physiol.* 154:343-401 (1993)].

In contrast to these observations, PCT Publication No. WO 94/03610 discloses a transmembrane PTP, termed PTP35, the steady state mRNA level of which was observed to be at a maximum in actively growing
25 cells. Little or no PTP35 mRNA expression was detected in confluent cell. This mode of regulation was also observed in mouse 3T3 cells. Thus, two RPTPs in the same cell type apparently participate in opposing processes, with one (PTP35) contributing to cellular growth and the other (the 35 kD PTP of Pallen and Tongs) contributing to cellular quiescence.

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Interestingly, transcription of Type II RPTP LAR messenger RNA has been demonstrated to be upregulated in confluent fibroblast cell culture [Longo, *et al.*, *J.Biol.Chem.* 268:26503-26511 (1993)]. LAR is proteolytically processed to generate a mature protein that is a complex of two non-covalently associated subunits, one containing the majority of the cell adhesion molecule-like extracellular domain [Yu, *et al.*, *Oncogene* 7:1051-1057 (1992); Streuli, *et al.*, *EMBO J.* 11:897-907 (1992)] and which is shed as cells approach confluence [Streuli, *et al.*, *EMBO J.* 11:897-907 (1992)]. These observations lead to speculation regarding PTP involvement in modulation of cytoskeletal integrity, as well as other related cellular phenomena such as transformation, tumor invasion, metastasis, cell adhesion, and leukocyte movement along and passage through the endothelial cell layer in inflammation. The therapeutic implications are enormous for modulators of PTP activity which are capable of regulating any or all of these cellular events.

There thus exists a need in the art to identify members of the PTP family of enzymes and to characterize these proteins in terms of their amino acid and encoding DNA sequences. Such information would provide for the large scale production of the proteins, allow for identification of cells which express the phosphatases naturally and permit production of antibodies specifically reactive with the phosphatases. Moreover, elucidation of the substrates, regulatory mechanisms, and subcellular localization of these PTPs would contribute to an understanding of normal cell growth and provide information essential for the development of therapeutic agents useful for intervention in abnormal and/or malignant cell growth.

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BRIEF DESCRIPTION OF THE INVENTION

As employed herein with respect to a protein tyrosine phosphatase, "density enhanced" denotes upregulated cellular mRNA transcription or translation and/or total cellular activity as a function of increased contact with neighboring cells.

5 In one aspect, the present invention provides purified and isolated polynucleotides (*e.g.*, DNA and RNA transcripts, both sense and anti-sense strands) encoding a Type III density enhanced protein tyrosine phosphatase enzymatic activity exemplified by the human phosphatase huDEP-1 and variants, including fragments, thereof (*i.e.*, fragments and deletion, addition or substitution analogs) which possess binding and/or immunological properties inherent to Type III density enhanced phosphatases. Preferred DNA molecules of the invention include cDNA, genomic DNA and wholly or partially chemically synthesized DNA molecules. A presently preferred polynucleotide is the DNA as set forth 10 in SEQ ID NO: 1, encoding the human DEP-1 polypeptide of SEQ ID NO: 2. Also provided are recombinant plasmid and viral DNA constructions (expression constructs) which include Type III density enhanced phosphatase encoding sequences, especially constructions wherein the Type III density enhanced phosphatase encoding sequence is operatively linked to a homologous or heterologous transcriptional regulatory element or elements. 20

As another aspect of the invention, prokaryotic or eukaryotic host cells transformed or transfected with DNA sequences of the invention are provided which express a Type III density enhanced phosphatase polypeptide or variants thereof. Host cells of the invention are particularly useful for large scale 25 production of Type III density enhanced phosphatase polypeptides, which can be isolated from either the host cell itself or the medium in which the host cell is grown. Host cells which express Type III density enhanced phosphatase polypeptides on the extracellular membrane surface are also useful as immunogens in the production of anti-Type III density enhanced phosphatase antibodies.

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Also provided by the present invention are purified and isolated Type III density enhanced phosphatase polypeptides, including fragments and variants thereof. A preferred Type III density enhanced phosphatase polypeptide is set forth in SEQ ID NO: 2. Novel Type III density enhanced phosphatase polypeptides and variant polypeptides may be obtained as isolates from natural sources, but are preferably produced by recombinant procedures involving host cells of the invention. Completely glycosylated, partially glycosylated and wholly un-glycosylated forms of the Type III density enhanced phosphatase polypeptide may be generated by varying the host cell selected for recombinant production and/or post-isolation processing. Variant Type III density enhanced phosphatase polypeptides of the invention may comprise water soluble and insoluble polypeptides including analogs wherein one or more of the amino acids are deleted or replaced: (1) without loss, and preferably with enhancement, of one or more biological activities or immunological characteristics specific for Type III density enhanced phosphatases; or (2) with specific disablement of a particular ligand/receptor binding or signalling function.

Also comprehended by the present invention are peptides, polypeptides, and other non-peptide molecules which specifically bind to Type III density enhanced phosphatases of the invention. Preferred binding molecules include antibodies (*e.g.*, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, anti-idiotypic antibodies, CDR-grafted antibodies and the like), counterreceptors (*e.g.*, membrane-associated and soluble forms) and other ligands (*e.g.*, naturally occurring or synthetic molecules), including those which competitively bind Type III density enhanced phosphatases in the presence of anti-Type III density enhanced phosphatase monoclonal antibodies and/or specific counterreceptors. Binding molecules are useful for purification of Type III density enhanced phosphatase polypeptides of the invention and for identifying cell types which express the polypeptide. Binding molecules are also useful for

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modulating (*i.e.*, inhibiting, blocking or stimulating) the *in vivo* binding and/or signal transduction activities of Type III density enhanced phosphatases.

Hybridoma cell lines which produce antibodies specific for Type III density enhanced phosphatases are also comprehended by the invention. Techniques for producing hybridomas which secrete monoclonal antibodies are well known in the art. Hybridoma cell lines may be generated after immunizing an animal with a purified Type III density enhanced phosphatase, or variants thereof, or cells which express a Type III density enhanced phosphatase or a variant thereof on the extracellular membrane surface. Immunogen cell types include cells which express a Type III density enhanced phosphatase *in vivo*, or transfected or transformed prokaryotic or eukaryotic host cells which normally do not express the protein *in vivo*.

The value of the information contributed through the disclosure of the DNA and amino acid sequences of human DEP-1 is manifest. In one series of examples, the disclosed human DEP-1 cDNA sequence makes possible the isolation of the human DEP-1 genomic DNA sequence, including transcriptional control elements. Transcriptional control elements comprehended by the invention include, for example, promoter elements and enhancer elements, as well as elements which contribute to repression, or downregulation, of mRNA transcription. Control elements of this type may be 5' DNA sequences or 3' DNA sequences with respect to the protein-encoding structural gene sequences, and/or DNA sequences located in introns. The 5' and/or 3' control elements may be proximal and/or distal the protein-encoding sequences of the structural gene. Identification of DNA sequences which modulate mRNA transcription in turn permits the identification of agents which are capable of effecting transcriptional modulation.

In another aspect, identification of polynucleotides encoding other Type III density enhanced phosphatases, huDEP-1 allelic variants and heterologous species (*e.g.*, rat or mouse) DNAs is also comprehended. Isolation

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of the huDEP-1 genomic DNA and heterologous species DNAs may be accomplished by standard nucleic acid hybridization techniques, under appropriately stringent conditions, using all or part of the DEP-1 DNA or RNA sequence as a probe to screen an appropriate library. Alternatively, polymerase chain reaction (PCR) using oligonucleotide primers that are designed based on the known nucleotide sequence can be used to amplify and identify other cDNA and genomic DNA sequences. Synthetic DNAs encoding Type III density enhanced phosphatase polypeptide, including fragments and other variants thereof, may be synthesized by conventional methods.

DNA sequence information of the invention also makes possible the development, by homologous recombination or "knockout" strategies [see, *e.g.*, Capecchi, *Science* 244:1288-1292 (1989)], of rodents that fail to express a functional Type III density enhanced phosphatase polypeptide or that express a variant Type III density enhanced phosphatase polypeptide. Such rodents are useful as models for studying the activities of Type III density enhanced phosphatases and modulators thereof *in vivo*.

DNA and amino acid sequences of the invention also make possible the analysis of Type III density enhanced phosphatase regions which actively participate in counterreceptor binding, as well as sequences which may regulate, rather than actively participate in, binding. Identification of motifs which participate in transmembrane signal transduction is also comprehended by the invention. Also comprehended is identification of motifs which determine subcellular localization of the immature and mature Type III density enhanced phosphatase proteins.

DNA of the invention is also useful for the detection of cell types which express Type III density enhanced phosphatase polypeptides. Identification of such cell types may have significant ramifications for development of therapeutic and prophylactic agents. Standard nucleic acid hybridization techniques which utilize *e.g.*, huDEP-1 DNA to detect corresponding RNAs, may

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be used to determine the constitutive level of Type III density enhanced phosphatase transcription within a cell as well as changes in the level of transcription in response to internal or external agents. Identification of agents which modify transcription, translation, and/or activity of Type III density enhanced phosphatases can, in turn, be assessed for potential therapeutic or prophylactic value. DNA of the invention also makes possible *in situ* hybridization of *e.g.*, huDEP-1 DNA to cellular RNA, to determine the cellular localization of Type III density enhanced phosphatase specific messages within complex cell populations and tissues.

Polynucleotides of the present invention also provide a method whereby substrate or other molecules which interact with Type III density enhanced phosphatases can be identified. A presently preferred method for identifying interacting molecules comprises the steps of: a) transforming or transfecting appropriate host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA-binding domain and an activating domain; b) an optional step of cotransforming or co-transfecting the same host cells with a protein tyrosine kinase (*e.g.*, v-src, c-src or the like) in order to phosphorylate potential interacting components and/or substrates introduced as in (d) below; c) expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of *e.g.*, a huDEP-1 isoform and either the DNA-binding domain or the activating domain of the transcription factor; d) expressing in the host cells a library of second hybrid DNA sequences encoding second fusions of part or all of putative DEP-1 isoform-binding proteins and either the activating domain or DNA binding domain of the transcription factor which is not incorporated in the first fusion; e) detecting binding of DEP-1 isoform-binding proteins to the DEP-1 isoform in a particular host cell by detecting the production of reporter gene product in the host cell; and f) isolating second hybrid DNA sequences encoding DEP-1 isoform-binding protein from the particular host cell. Variations of the method altering

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the order in which *e.g.*, the huDEP-1 isoforms and putative huDEP-1 isoform-binding proteins are fused to transcription factor domains, either at the amino terminal or carboxy terminal end of the transcription factor domains, are contemplated. In a preferred method, the promoter is the ADHI promoter, the
5 DNA-binding domain is the *lexA* DNA-binding domain, the activating domain is the GAL4 transactivation domain, the reporter gene is the *lacZ* gene and the host cell is a yeast host cell. Those of ordinary skill in the art will readily envision that any of a number of other reporter genes and host cells are easily amenable to this technique. Likewise, any of a number of transcription factors with distinct
10 DNA binding and activating domains can be utilized in this procedure, either with both the DNA binding and activating domains derived from the same transcription factor, or from different, but compatible transcription factors. As another variation of this method, mutant DEP-1 polypeptides, wherein a cysteine residue in the catalytic domain has been substituted with a serine residue, can be
15 employed in this technique. Mutations of this type have been demonstrated with other phosphatases to recognize and bind substrates, but do not dephosphorylate the substrate since the phosphatase is inactive as a result of the mutation.

An alternative identification method contemplated by the invention for detecting proteins which bind to a Type III density enhanced phosphatase
20 isoform comprises the steps of: a) transforming or transfecting appropriate host cells with a hybrid DNA sequence encoding a fusion between a putative Type III density enhanced phosphatase isoform-binding protein and a ligand capable of high affinity binding to a specific counterreceptor; b) expressing the hybrid DNA sequence in the host cells under appropriate conditions; c) immobilizing fusion
25 protein expressed by the host cells by exposing the fusion protein to the specific counterreceptor in immobilized form; d) contacting a Type III density enhanced phosphatase isoform with the immobilized fusion protein; and e) detecting the Type III density enhanced phosphatase isoform bound to the fusion protein using a reagent specific for the Type III density enhanced phosphatase isoform.

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Presently preferred ligands/counterreceptor combinations for practice of the method are glutathione-S-transferase/glutathione, hemagglutinin/hemagglutinin-specific antibody, polyhistidine/nickel and maltose-binding protein/amylose.

Additional methods to identify proteins which specifically interact
5 with Type III density enhanced phosphatase (*i.e.*, substrates, ligands, modulators, *etc.*) are also contemplated by the invention. In one example, purified and isolated Type III density enhanced phosphatase polypeptide (*e.g.*, huDEP-1 polypeptide) can be covalently coupled to an immobilized support (*i.e.*, column resins, beads, *etc.*) and incubated with cell lysates to permit protein/protein
10 interactions. Proteins which interact with the immobilized DEP-1 polypeptide can then be eluted from the support with gradient washing techniques which are standard in the art.

As another example, protein overlay techniques can be employed. DNA from cells which either express *e.g.*, huDEP-1 or express polypeptides
15 which can modulated or bind to huDEP-1, can be isolated and a library constructed by standard methods. This library can then be expressed in a heterologous cell line and resulting colonies transferred to an immobilizing support. Expressed proteins from these colonies are then contacted with DEP-1 and incubated under appropriate conditions to permit DEP-1/protein interactions.
20 The resulting Type III density enhanced phosphatase/protein complexes formed can be detected by incubation with a specific Type III density enhanced phosphatase antibody. Colonies which interact with the specific antibody contain DNA encoding a protein which interacts with the Type III density enhanced phosphatase. Alternatively, cell or tissue lysates may be employed in this
25 technique, using cells or tissues which normally express DEP-1, or cells which have been previously transfected or transformed with DEP-1 encoding DNA.

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BRIEF DESCRIPTION OF THE DRAWING

Numerous other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description thereof, reference being made to the drawing wherein:

5 Figures 1A through 1B are photographs of Northern blot analysis autoradiograms; and

 Figures 2 shows the density-dependent expression of DEP-1.

DETAILED DESCRIPTION OF THE INVENTION

10 The present invention is illustrated by the following examples relating to the isolation and characterization of genes encoding Type III density enhanced phosphatase polypeptides. Example 1 relates to the isolation of cDNA encoding human DEP-1. Example 2 discusses the tissue distribution of huDEP-1 as determined by Northern blot analysis. Example 3 addresses the generation of antibodies specific for DEP-1 and fragments thereof. Example 4 demonstrates
15 expression of a huDEP-1 cDNA clone in COS cells. Example 5 relates to detection of endogenous expression of huDEP-1 in fibroblast cells. Example 6 addresses expression of huDEP-1 as a function of cell culture density. Example 7 relates to identification of ligands of huDEP-1. Example 8 discusses identification of modulators and substrates of huDEP-1 activity. Example 9
20 details characterization of the genomic huDEP-1 DNA.

Example 1**Isolation and Characterization of huDEP-1 cDNA**

 In initial efforts to isolate cDNA encoding a novel human phosphatase regulated by a cell density-dependent mechanism, PCR primers were
25 synthesized based on conserved amino acid sequences common to many previously identified phosphatases. These primers were then used to amplify

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polynucleotides from a cDNA library, the resulting amplification products were sequenced, and these sequences compared to previously reported DNA sequences.

Degenerate primers, corresponding to conserved PTP amino acid sequences set out in SEQ ID NO: 3 and SEQ ID NO: 4, were synthesized and used to prime a PCR with a HeLa cell cDNA library as template.

KCAQYWP SEQ ID NO: 3

HCSAGIG SEQ ID NO: 4

The corresponding primers used in the PCR reaction are set forth in SEQ ID NO: 5 and SEQ ID NO: 6, respectively, employing nucleotide symbols according to 37 U.S.C. § 1.882.

5'-AARTGYGCNCARTAYTGGCC-3' SEQ ID NO: 5

3'-GTRACRTCRCGNCCITADCC-5' SEQ ID NO: 6

Sequencing of seventy-seven independent subclones revealed seven distinct sequences, six of which corresponded to PTPs for which DNA sequences had previously been published, and included PTP1B [Tonks, *et al.*, *J.Biol.Chem* 263:6722-6730 (1988)], TCPTP [Cool, *et al.*, *Proc.Natl.Acad.Sci(USA)* 86:5257-5261 (1989)], RPTP α [Krueger, *et al.*, *EMBO J.* 9:3241-3252 (1990)], LAR [Streuli, *et al.*, *J.Exp.Med.* 168:1523-1530 (1988)], PTPH1 [Yang and Tonks, *Proc.Natl.Acad.Sci.(USA)* 88:5949-5953 (1991)], and PTP μ [Gebbink, *et al.*, *FEBS Lett.* 290:123-130 (1991)]. The seventh clone was determined to comprise a unique 300 bp PCR fragment and was used to screen an oligo-dT-primed HeLa cell cDNA library (Stratagene, La Jolla, CA) in an effort to isolate a corresponding full-length cDNA. Approximately 1.8×10^6 phage plaques were screened as previously described [Yang and Tonks, *Proc.Natl.Acad.Sci.(USA)* 88:5949-5953 (1991)] and twenty-four positive clones were identified. The largest insert, a 5.1 kb cDNA, was cloned into pUC119, sequenced by the dideoxy chain termination method, and found to contain an open reading frame of 4011 nucleotides encoding a novel receptor-like PTP of 1337 amino acids. The DNA sequence of the 5.1 kb insert is set out in SEQ ID NO: 1, and its predicted amino

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acid sequence is set out in SEQ ID NO: 2. This human density-enhanced PTP was designated huDEP-1.

The proposed initiating ATG codon of the huDEP-1 gene is flanked by a purine (G) at the -3 position and is thus in agreement with the Kozak rules for initiation [Kozak, *J. Cell Biol.* 108:229-241 (1989)]. There is an in-frame stop codon approximately 290 bp upstream of the predicted initiation site, and the initiating ATG is followed by a hydrophobic region that may serve as a signal sequence. Based on the statistical analysis of known cleavage sites for the signal peptidase [von Heijne, *Nuc. Acids Res.* 14:4683-4690 (1986)], the amino terminus of the mature huDEP-1 polypeptide is assigned to Gly³⁷. A second hydrophobic region is found between amino acids 977 and 996, and is followed by a stretch of predominantly basic residues, characteristic of a stop transfer sequence. Therefore, an extracellular region of 940 amino acids and an intracellular portion of 341 amino acids are predicted for the mature huDEP-1 protein. The extracellular domain comprises eight FNIII domains, and thirty-three potential sites for N-linked glycosylation are predicted. Thus, huDEP-1 conforms to the RPTP Type III topography according to the nomenclature of Fischer *et al.*, *supra*. Unlike most RPTPs which possess a tandem repeat of catalytic domains, the cytoplasmic region contains a single catalytic domain spanning amino acid residues 1060 through 1296. Human DEP-1 is therefore representative of an expanding group of RPTPs with a single catalytic domain that includes PTP β [Krueger, *et al.*, *EMBO J.* 9:3241-3252 (1990)], DPTPIOD of *Drosophila* [Tian, *et al.*, *Cell* 76:675-685 (1991); Yang, *et al.*, *Cell* 67:661-673 (1991)], DPTP4E of *Drosophila* [Oon, *et al.*, *J. Biol. Chem.* 268:23964-23971 (1993)], and the recently described SAP-I enzyme [Matozaki, *et al.*, *J. Biol. Chem.* 269:2075-2081 (1994)]. Amino acid sequence comparison of the catalytic domain of huDEP-1 with other PTP domains revealed huDEP-1 is most closely related to PTP β and SAP-1. The sequence includes several Ser-Pro motifs, as well as potential sites for phosphorylation by casein kinase II.

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Example 2

Northern Analysis of huDEP-1 Tissue Distribution

Because the expression of PTPs has previously been demonstrated to be ubiquitous in eukaryotes, various human tissues were analyzed in order to determine the relative degree of huDEP-1 mRNA expression.

RNA Multi Tissue Northern blot filters (Clontech, Palo Alto, CA), containing immobilized RNA from various human tissues, were probed with a 1.6 kb *HindIII/EcoRI* fragment of the huDEP-1 cDNA previously radiolabeled to a specific activity of 1.5×10^6 cpm/ng using a Megaprime DNA labeling kit (Amersham, Arlington Heights, IL). This probe represented the entire length of the isolated huDEP-1 cDNA. Hybridization was performed for 16 hours at 65°C in a hybridization buffer containing 0.5 M Na_2HPO_4 , 7% SDS, 1 mM EDTA, and labeled probe at a concentration of 10^6 cpm/ml. Filters were then washed 5 times at 65°C in 40 mM Na_2HPO_4 , 1% SDS, and 1 mM EDTA. The membrane was then subjected to autoradiography. The results are presented in Figures 1A and 1B, wherein the human tissue source of immobilized RNA is as follows. In Figure 1A, RNA in lane 2 is from heart, lane 3 from brain, lane 4 from placenta, lane 5 from lung, lane 6 from liver, lane 7 from skeletal muscle, lane 8 from kidney, and lane 9 from pancreas. In Figure 1B, RNA in lane 2 is from spleen, lane 3 from thymus, lane 4 from prostate, lane 5 from testis, lane 6 from ovary, lane 7 from small intestines, lane 8 from colon, and lane 9 from peripheral blood leukocyte.

Northern analysis indicated that huDEP-1 is expressed in most tissues analyzed, with particularly high mRNA levels detected in placenta, kidney, spleen and peripheral blood leukocytes.

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Example 3

Generation of huDEP-1 Polyclonal Antibodies

Two peptides, unique to huDEP-1 and corresponding to amino acid residues 1297 through 1315 and residues 1321 through 1334 in SEQ ID NO: 2 (downstream from the catalytic region) were synthesized with an additional amino terminal cysteine residue and conjugated to rabbit serum albumin (RSA) with *m*-maleimido benzoic acid N-hydroxysuccinimide ester (MBS)(Pierce, Rockford, IL). Immunization protocols with these peptides were performed by Cocalico Biologicals (Reamstown, PA). Initially, a pre-bleed of the rabbits was performed prior to immunization. The first immunization included Freund's complete adjuvant and 500 μ g conjugated peptide or 100 μ g purified peptide. All subsequent immunizations, performed four weeks after the previous injection, included Freund's incomplete adjuvant with the same amount of protein. Bleeds were conducted seven to ten days after the immunizations.

For affinity purification of the antibodies, huDEP-1 peptide conjugated to RSA with MBS, was coupled to CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden). Antiserum was diluted 10-fold in 10 mM Tris-HCl, pH 7.5, and incubated overnight with the affinity matrix. After washing, bound antibodies were eluted from the resin with 100 mM glycine, pH 2.5.

The antibody generated against conjugated amino acid residues 1297 through 1315 was designated anti-CSH-241, and the antibody raised against the conjugated peptide corresponding to amino acid residues 1321 through 1334 was designated anti-CSH-243.

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Example 4

Expression of huDEP-1 by Transfected Host Cells

To study the protein product of the huDEP-1 cDNA, the 5.1 kb *EcoRI* insert was cloned into the expression vector pMT2 [Sambrook, *et al.*,
5 Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press
(1989) pp 16.17-16.22] and transfected into COS cells grown in DMEM
supplemented with 10% FCS. Transfections were performed employing calcium
phosphate techniques [Sambrook, *et al* (1989) pp. 16.32-16.40, *supra*] and cell
lysates were prepared forty-eight hours after transfection from both transfected
10 and untransfected COS cells. Lysates were subjected to analysis by
immunoblotting using anti-CSH-243 antibody, and PTP assays of immune
complexes as addressed below.

In immunoblotting experiments, preparation of cell lysates and
electrophoresis were performed. Protein concentration was determined using
15 BioRad protein assay solutions. After semi-dry electrophoretic transfer to
nitro-cellulose, the membranes were blocked in 500 mM NaCl, 20 mM Tris, pH
7.5, 0.05% Tween-20 (TTBS) with 5% dry milk. After washing in TTBS and
incubation with secondary antibodies (Amersham), enhanced chemiluminescence
(ECL) protocols (Amersham) were performed as described by the manufacturer
20 to facilitate detection.

For immune complex PTP assays, 60 μ g of cell lysate were
immunoprecipitated with 20 μ l of anti-CSH-243 antisera or preimmune rabbit
serum bound to 25 μ l of Protein-A Sepharose (Pharmacia). After overnight
incubation at 4° C, the immune complexes were washed three times in washing
25 buffer (1% Triton X-100, 150 mM NaCl, 20 mM Hepes, pH 7.5, 5 μ g/ml
aprotinin, 5 μ g/ml leupeptin, 1 mM benzamidine, and 1 mM DTT) and once in
assay buffer (25 mM imidazole, pH 7.2, 0.5 mg/ml BSA, and 1 mM DTT).
Protein-A Sepharose immune complexes were then resuspended in 150 μ l of assay
buffer and assayed for PTP activity as triplicates. Assays were performed for 6

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minutes at 30° C in a total volume of 60 μ l using 3 μ M [32 P-Tyr]-reduced carboxymethylated (RCM) lysozyme as substrate [Flint, *et al.*, *EMBO J.* 12:1937-1946 (1993)].

5 Affinity-purified anti-CSH-243 antibodies specifically detected a protein of 180 kD molecular weight in lysates from transfected cells. Furthermore, when immune complexes were analyzed for PTP activity, almost 10-fold higher activity was detected in anti-CSH-243 immune complexes from the transfected cells compared to the untransfected cells. This PTP activity was largely absent in immune complexes derived from immunoprecipitations with blocked antiserum or preimmune serum. It was concluded that the huDEP-1 10 cDNA encodes a 180 kD protein with intrinsic PTP activity.

Example 5

Endogenous Expression of huDEP-1

15 To characterize endogenously expressed huDEP-1, lysates from different cell lines including CEM (ATCC CCL 119), HeLa (ATCC CCL 2), 293 (ATCC CRL 1573), Jurkat (ATCC TIB 152), K562 (ATCC CCL243), HL60 (ATCC CCL 240), WI38 (ATCC CCL 75) and AG 1518 (Coriell Cell Repositories, Camden, NJ) were analyzed by immunoblotting with antibody anti-CSH-243 as described in Example 4.

20 WI38 cells, a diploid fetal lung fibroblast-like cell line with finite life span, showed the highest expression. Similar levels of expression were also detected in AG 1518 foreskin fibroblast cells.

25 To further examine the expression of huDEP-1, lysates from metabolically labeled cells were analyzed by immunoprecipitation and SDS-gel electrophoresis. Confluent cultures of WI38 and AG 1518 cells were metabolically labeled for four hours in methionine-free DMEM supplemented with 1 mg/ml bovine serum albumin (BSA) and 0.15 mCi/ml Translabel (ICN, Costa Mesa, CA). Cells were lysed in 0.5% DOC, 0.5% Triton X-100, 150 mM NaCl,

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20 mM Hepes, pH 7.5, 5 μ g/ml aprotinin, 5 μ g/ml leupeptin, 1 mM benzamidine, 1 mM DTT (lysis buffer) and lysates were centrifuged at 15,000 x g for 15 minutes. Lysates corresponding to approximately 2×10^6 cells were then incubated with 20 μ l of anti-CSH-243 or anti-CSH-243. After incubation for four hours at 4° C, 50 μ l of a 1:1 Protein-A-Sepharose slurry was added to bind the protein/antibody complexes and incubation continued for 60 minutes. Immune complexes adsorbed to the Protein-A-Sepharose were collected by centrifugation and washed three times in 1% Triton X-100, 150 mM NaCl, 20 mM Hepes, pH 7.5, 5 μ g/ml aprotinin, 5 μ g/ml leupeptin, 1 mM benzamidine, 1 mM DTT (washing buffer) and once in 20 mM Tris, pH 7.5. Samples were eluted from the resin by incubation at 95° C for 3 minutes in reducing SDS-sample buffer and analyzed by SDS-gel electrophoresis on 7% gels, followed by fluorography.

In both WI38 and AG 1518 cells, a protein of 180 kD was recognized specifically by the unblocked antisera. Anti-CSH-243 antisera immunoprecipitation with WI38 cell lysate also yielded significantly higher amounts (approximately 10 to 20 fold higher) of activity than precipitations with pre-immune serum or antiserum that had been previously incubated with 200 μ g/ml of peptide-conjugate.

It appears that huDEP-1 is a phosphoprotein *in vivo* because the fact that the anti-CSH-243 antibody was capable of immunoprecipitating a 180 kD [32 P]-labeled protein from a cell lysate of WI38 cells which had been metabolically labelled with [32 P]-inorganic phosphate.

Example 6

Cell Density-Dependent Expression and Activity of huDEP-1.

WI38 cell lysates from sparse (less than 7,000 cells /cm²) or dense (more than 25,000 cells/cm²) cultures were compared for levels of expressed huDEP-1 protein by immunoblotting with anti-CSH-243 antibody as described in Example 4. A dramatic, ten- to twenty-fold increase in huDEP-1 expression was

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detected in dense cell cultures as shown in Figure 2. Since 3 μ g of total cell lysate from more confluent culture gave a relatively strong signal, and 15 μ g of lysates from sparse cultures were below detection, it was estimated that at least 10-fold higher amounts of huDEP-1 are present in cells from dense cultures. Similar results were obtained with anti-CSH-241. When the amounts of PTP1B in cell lysates from sparse and dense cells were compared using an anti-PTP1B monoclonal antibody FG6 (Oncogene Science, Uniondale, NY), no difference was observed. The observed effects on huDEP-1 expression are not restricted to WI38 cells as similar results were obtained in AG 1518 cells.

In order to determine if enzyme activity was also regulated by a density-dependent mechanism, huDEP-1 and PTP1B immune complexes and total cell lysates from both sparse and dense WI38 and AG 1518 cell cultures were also analyzed for phosphatase activity using the PTP assay. For immune complex PTP assays, 60 μ g of cell lysate were immunoprecipitated with 20 μ l of anti-CSH-243 antisera (with or without pretreatment with antigen) or preimmune serum bound to 25 μ l of Protein-A Sepharose. After incubation overnight at 4° C, immune complexes were washed three times in washing buffer and once in 25 mM imidazole, pH 7.2, 0.5 mg/ml BSA, 1 mM DTT (assay buffer). Protein-A-Sepharose immune complexes were then suspended in 150 μ l of assay buffer and assayed for PTP activity as triplicates. Assays were performed for 6 minutes at 30° C in a total volume of 60 μ l using 3 μ M [32 P-Tyr] RCM lysozyme as substrate [Flint, *et al.*, *supra*].

In agreement with the increased huDEP-1 protein expression demonstrated in the immunoblotting experiments, huDEP-1 enzyme activity also increased in the dense cell cultures. The observed increase in activity in huDEP-1/CSH-243 immunoprecipitates from dense cultures (approximately two- to three-fold) was not as great as the observed increase in protein expression in dense cultures, most likely due to incomplete precipitation of all of the PTP using

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anti-CSH-243 antisera. No difference was observed in activity of PTP1B/FG6 immunoprecipitates or total cell lysates from sparse and dense cell cultures.

Finally, to investigate the kinetics of the density-dependent upregulation of huDEP-1 expression, lysates of WI38 and AG 1518 cells at intermediate cell densities were included in the immunoblotting analysis. The highest expression was found in cells at saturation density, however, at intermediate densities an increase in expression with respect to sparse cell cultures was also observed. Thus, the upregulation of huDEP-1 expression appears to be initiated prior to saturation density and not a result of growth arrest.

While the precise mechanism by which huDEP-1 expression is induced remains unclear, the demonstration that expression was induced in two distinct cell lines as cells approach confluence suggests involvement of huDEP-1 in promoting net dephosphorylation of proteins, countering the effects of growth promoting PTK activity. This possibility, in combination with the broad distribution of huDEP-1 expression, suggests that huDEP-1 may be involved in a general mechanism for contact inhibition of cell growth.

Example 7

Identification of DEP-1 Ligands

The possibility that DEP-1 functions as an adhesion molecule will be tested using the Sf9 cell system [Brady-Kalnay, *et al.*, *J. Cell Biol.* 122:961-972 (1993)] following transfection with DEP-1 cDNA. In addition to studies following transient expression, stable cell lines overexpressing DEP-1 will be generated.

If DEP-1 functions as an adhesion molecule, the extracellular counterreceptor(s) will be identified. One possibility is that, like PTP μ , DEP-1 binding is homophilic, where one DEP-1 molecule binds another DEP-1 molecule on an adjacent cell. Alternatively, DEP-1 specifically recognize a non-DEP-1 molecule in a heterophilic binding mechanism.

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In addition, a number of deletion and site-directed mutagenesis strategies well known in the art will be applied to identify the important segments in the protein that confer binding specificity. Analysis of 2D gels of proteins that react with anti-phosphotyrosine antibodies, for example monoclonal antibody
5 4G10 (UBI, Lake Placid, NY), will be used to initiate studies as to the effect on activity of engagement of the extracellular segment of the PTP in either homophilic binding interactions or antibody binding.

Use of "epitope" library technology [Scott and Smith, *Science* 249:386-390 (1990)] will be employed to identify peptide sequences that interact
10 with DEP-1. This approach will prove particularly useful in the search for ligands for DEP-1 whose extracellular segment, comprising multiple FNIII repeats, may bind low M_r factors.

Protein:protein interactions have previously been reported for FNIII sequences and specific binding proteins, and this information will be utilized in
15 several approaches to identify proteins which specifically interact with the extracellular domain of DEP-1. Specifically, protein:protein interactions will be investigated in cell "panning" experiments [Seed and Aruffo, *Proc. Natl. Acad. Sci. (USA)* 84:3365-3369 (1987)], gel overlay assays [Hirsch, *et al.*, *J. Biol. Chem.* 267:2131-2134 (1992); Carr and Scott, *Trends in Biochemical*
20 *Sci.* 17:246-249 (1992)], band shift analysis [Carr, *et al.*, *J. Biol. Chem.* 267:13376-13382 (1992)], affinity chromatography, screening of expression libraries [Young and Davis, *Proc. Natl. Acad. Sci. (USA)* 80:1194-1198 (1983)], etc.

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Example 8

Identification of Modulators/Substrates of DEP-1

Potential substrates of predicted physiological relevance will be tested for activity against the catalytic domain *in vitro*.

5 In addition, yeast screening systems [Fields and Song, *Nature* 340:245-246 (1989); Yang, *et al.*, *Science* 257:6810682 (1992); Vojtek, *et al.*, *Cell* 74:205-214 (1993)] will be utilized, particularly with reference to co-expression with a protein tyrosine kinase, for example, v-src or c-src, to isolate proteins with the capacity to regulate DEP-1 activity.

10 In a further attempt to identify substrates for DEP-1, a mutant form in which the cysteinyl residues of the active center has been replaced by serine will be expressed. Recent studies suggest that substrates bind to and remain complexed with the inactive phosphatase. The mutant PTP is capable of binding substrate molecules but traps them in a "dead end" complex that can be isolated
15 by standard immunoprecipitation techniques [Sun, *et al.*, *Cell* 75:487-493 (1993)]. Potential substrates may be co-immunoprecipitated with the mutant PTP from ³⁵S-labeled cells. Alternatively, wild-type, or native, DEP-1 enzyme may be utilized in this technique. Initial studies in this direction may make use of chimeric molecules, for which antibodies to the extracellular growth factor binding segment
20 are commercially available, while antibodies are raised to the bona fide DEP-1 sequences.

Example 9

Characterization of the Genomic DEP-1 Gene

25 Isolation of the cDNA sequences for DEP-1 will permit the isolation and purification of the corresponding genomic sequences for DEP-1. In preliminary work, it has been demonstrated that huDEP-1 mapped to human chromosome 11p, band 11.2 or the interface of 11.2 and 11.3. Isolation of these genomic DEP-1 sequences will permit the identification of putative regulatory

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sequences for DEP-1 transcription, and presumably identification of *trans*-acting transcriptional modulators of DEP-1 expression. In addition, isolation and purification of the human genomic clone will permit screening of libraries in other species to determine if homologous counterparts exist in the species.

- 5 Identification of a homologous counterpart in mice will be of particular importance because of the possibility of generating a knockout strain. Mouse strains which do not express a particular protein are of considerable importance in that they permit determination of indications associated with absence of the protein in a living animal.

- 10 While the present invention has been described in terms of specific methods and compositions, it is understood that variations and modifications will occur to those skilled in the art. Therefore, only such limitations as appear in the claims should be placed on the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANTS: Tonks, Nicholas K. and Östman, Arne
- (ii) TITLE OF INVENTION: Density Enhanced Protein Tyrosine Phosphatase
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
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 - (E) COUNTRY: United States of America
 - (F) ZIP: 60606
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Borun, Michael F.
 - (B) REGISTRATION NUMBER: 25,447
 - (C) REFERENCE/DOCKET NUMBER: 27866/31954
- (ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5117 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 350..4364

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCCCAGCCGC ATGACGCGCG GAGGAGGCAG CGGGACCAGC GCGGGAGCCG GGACCGGGTA	60
GCCGCGCGCT GGGGGTGGGC GCCGCTCGCT CCGCCCCGCG AAGCCCCTGC GCGCTCAGGG	120

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ACGCGGCCCC	CCCGCGGCAG	CCGCGCTAGG	CTCCGGCGTG	TGGCCGCGGC	CGCCGCCGCG	180
CTGCCATGTC	TCCGGGAAG	CCGGGGCGGG	CGGAGCGGGG	ACGAGGCGGA	CCGGCTGGCG	240
GAGGAGGAGG	CGAAGGAGAC	GGCAGGAGGC	GGCGACGACG	GTGCCCCGGC	TCGGGCGCAC	300
GGCGGGGCCC	GATTCGCGCG	TCCGGGGCAC	GTTCCAGGGC	GCGCGGGGC	ATG AAG Met Lys 1	355
CGG GCG GCG CGG GAG GCG CGG CTG CCT CCG CGC TCG CCC GGG CTG CGC	403					
Pro Ala Ala Arg Glu Ala Arg Leu Pro Pro Arg Ser Pro Gly Leu Arg						
5 10 15						
TGG GCG CTG CCG CTG CTG CTG CTG CTG CTG CGC CTG GGC CAG ATC CTG	451					
Trp Ala Leu Pro Leu Leu Leu Leu Leu Leu Arg Leu Gly Gln Ile Leu						
20 25 30						
TGC GCA GGT GGC ACC CCT AGT CCA ATT CCT GAC CCT TCA GTA GCA ACT	499					
Cys Ala Gly Gly Thr Pro Ser Pro Ile Pro Asp Pro Ser Val Ala Thr						
35 40 45 50						
GTT GCC ACA GGG GAA AAT GGC ATA ACG CAG ATC AGC AGT ACA GCA GAA	547					
Val Ala Thr Gly Glu Asn Gly Ile Thr Gln Ile Ser Ser Thr Ala Glu						
55 60 65						
TCC TTT CAT AAA CAG AAT GGA ACT GGA ACA CCT CAG GTG GAA ACA AAC	595					
Ser Phe His Lys Gln Asn Gly Thr Gly Thr Pro Gln Val Glu Thr Asn						
70 75 80						
ACC AGT GAG GAT GGT GAA AGC TCT GGA GCC AAC GAT AGT TTA AGA ACA	643					
Thr Ser Glu Asp Gly Glu Ser Ser Gly Ala Asn Asp Ser Leu Arg Thr						
85 90 95						
CCT GAA CAA GGA TCT AAT GGG ACT GAT GGG GCA TCT CAA AAA ACT CCC	691					
Pro Glu Gln Gly Ser Asn Gly Thr Asp Gly Ala Ser Gln Lys Thr Pro						
100 105 110						
AGT AGC ACT GGG CCC AGT CCT GTG TTT GAC ATT AAA GCT GTT TCC ATC	739					
Ser Ser Thr Gly Pro Ser Pro Val Phe Asp Ile Lys Ala Val Ser Ile						
115 120 125 130						
AGT CCA ACC AAT GTG ATC TTA ACT TGG AAA AGT AAT GAC ACA GCT GCT	787					
Ser Pro Thr Asn Val Ile Leu Thr Trp Lys Ser Asn Asp Thr Ala Ala						
135 140 145						
TCT GAG TAC AAG TAT GTA GTA AAG CAT AAG ATG GAA AAT GAG AAG ACA	835					
Ser Glu Tyr Lys Tyr Val Val Lys His Lys Met Glu Asn Glu Lys Thr						
150 155 160						
ATT ACT GTT GTG CAT CAA CCA TGG TGT AAC ATC ACA GGC TTA CGT CCA	883					
Ile Thr Val Val His Gln Pro Trp Cys Asn Ile Thr Gly Leu Arg Pro						
165 170 175						
GCG ACT TCA TAT GTA TTC TCC ATC ACT CCA GGA ATA GGC AAT GAG ACT	931					
Ala Thr Ser Tyr Val Phe Ser Ile Thr Pro Gly Ile Gly Asn Glu Thr						
180 185 190						
TGG GGA GAT CCC AGA GTC ATA AAA GTC ATC ACA GAG CCG ATC CCA GTT	979					
Trp Gly Asp Pro Arg Val Ile Lys Val Ile Thr Glu Pro Ile Pro Val						
195 200 205 210						

- 29 -

TCT	GAT	CTC	CGT	GTT	GCT	CAC	GGG	TGT	GAG	GAA	GGC	TGC	TCT	CTC	TCC	1027
Ser	Asp	Leu	Arg	Val	Ala	His	Gly	Cys	Glu	Glu	Gly	Cys	Ser	Leu	Ser	
				215					220					225		
TGG	AGC	AAT	GGC	AAT	GGC	ACC	GCC	TCC	TGC	CGG	GTT	CTT	CTT	GAA	AGC	1075
Trp	Ser	Asn	Gly	Asn	Gly	Thr	Ala	Ser	Cys	Arg	Val	Leu	Leu	Glu	Ser	
			230					235					240			
ATT	GGA	AGC	CAT	GAG	GAG	TTG	ACT	CAA	GAC	TCA	AGA	CTT	CAG	GTC	AAT	1123
Ile	Gly	Ser	His	Glu	Glu	Leu	Thr	Gln	Asp	Ser	Arg	Ile	Gln	Val	Asn	
			245				250					255				
ATC	TCG	GAC	CTG	AAG	CCA	GGG	GTT	CAA	TAC	AAC	ATC	AAC	CCG	TAT	CTT	1171
Ile	Ser	Asp	Leu	Lys	Pro	Gly	Val	Gln	Tyr	Asn	Ile	Asn	Pro	Tyr	Leu	
			260			265					270					
CTA	CAA	TCA	AAT	AAG	ACA	AAG	GGA	GAC	CCC	TTG	GCA	CAG	AAG	GTG	GCT	1219
Leu	Gln	Ser	Asn	Lys	Thr	Lys	Gly	Asp	Pro	Leu	Ala	Gln	Lys	Val	Ala	
					280					285					290	
TGG	ATG	CCA	GCA	ATA	CAG	AGA	GAA	GCC	GGG	CAG	GGA	GCC	CCA	CCG	CCC	1267
Trp	Met	Pro	Ala	Ile	Gln	Arg	Glu	Ala	Gly	Gln	Gly	Ala	Pro	Pro	Pro	
				295				300						305		
CTG	TGC	ATG	ATG	AGT	CCC	TTC	GTG	GGA	CCT	GTG	GAC	CCA	TCC	TCC	GGC	1315
Leu	Cys	Met	Met	Ser	Pro	Phe	Val	Gly	Pro	Val	Asp	Pro	Ser	Ser	Gly	
				310			315						320			
CAG	CAG	TCC	CGA	GAC	ACG	GAA	GTC	CTG	CTT	GTC	GGG	TTA	GAG	CCT	GGC	1363
Gln	Gln	Ser	Arg	Asp	Thr	Glu	Val	Leu	Leu	Val	Gly	Leu	Glu	Pro	Gly	
			325				330					335				
ACC	CGA	TAC	AAT	GCC	ACC	GTT	TAT	TCC	CAA	GCA	GCG	AAT	GGC	ACA	GAA	1411
Thr	Arg	Tyr	Asn	Ala	Thr	Val	Tyr	Ser	Gln	Ala	Ala	Asn	Gly	Thr	Glu	
						345					350					
GGA	CAG	CCC	CAG	GCC	ATA	GAG	TTC	AGG	ACA	AAT	GCT	ATT	CAG	GTT	TTT	1459
Gly	Gln	Pro	Gln	Ala	Ile	Glu	Phe	Arg	Thr	Asn	Ala	Ile	Gln	Val	Phe	
					360					365					370	
GAC	GTC	ACC	GCT	GTG	AAC	ATC	AGT	GCC	ACA	AGC	CTG	ACC	CTG	ATC	TGG	1507
Asp	Val	Thr	Ala	Val	Asn	Ile	Ser	Ala	Thr	Ser	Leu	Thr	Leu	Ile	Trp	
				375				380						385		
AAA	GTC	AGC	GAT	AAC	GAG	TCG	TCA	TCT	AAC	TAT	ACC	TAC	AAG	ATA	CAT	1555
Lys	Val	Ser	Asp	Asn	Glu	Ser	Ser	Ser	Asn	Tyr	Thr	Tyr	Lys	Ile	His	
			390					395					400			
GTG	GCG	GGG	GAG	ACA	GAT	TCT	TCC	AAT	CTC	AAC	GTC	AGT	GAG	CCT	CGC	1603
Val	Ala	Gly	Glu	Thr	Asp	Ser	Ser	Asn	Leu	Asn	Val	Ser	Glu	Pro	Arg	
			405				410					415				
GCT	GTC	ATC	CCC	GGA	CTC	CGC	TCC	AGC	ACC	TTC	TAC	AAC	ATC	ACA	GTG	1651
Ala	Val	Ile	Pro	Gly	Leu	Arg	Ser	Ser	Thr	Phe	Tyr	Asn	Ile	Thr	Val	
						425					430					
TGT	CCT	GTC	CTA	GGT	GAC	ATC	GAG	GGC	ACG	CCG	GGC	TTC	CTC	CAA	GTG	1699
Cys	Pro	Val	Leu	Gly	Asp	Ile	Glu	Gly	Thr	Pro	Gly	Phe	Leu	Gln	Val	
					440					445					450	
CAC	ACC	CCC	CCT	GTT	CCA	GTT	TCT	GAC	TTC	CGA	GTG	ACA	GTG	GTC	AGC	1747

- 30 -

His	Thr	Pro	Pro	Val	Pro	Val	Ser	Asp	Phe	Arg	Val	Thr	Val	Val	Ser	
				455					460						465	
ACG	ACG	GAG	ATC	GGC	TTA	GCA	TGG	AGC	AGC	CAT	GAT	GCA	GAA	TCA	TTT	1795
Thr	Thr	Glu	Ile	Gly	Leu	Ala	Trp	Ser	Ser	His	Asp	Ala	Glu	Ser	Phe	
			470					475					480			
CAG	ATG	CAT	ATC	ACA	CAG	GAG	GGA	GCT	GGC	AAT	TCT	CGG	GTA	GAA	ATA	1843
Gln	Met	His	Ile	Thr	Gln	Glu	Gly	Ala	Gly	Asn	Ser	Arg	Val	Glu	Ile	
		485					490					495				
ACC	ACC	AAC	CAA	AGT	ATT	ATC	ATT	GGT	GGC	TTG	TTC	CCT	GGA	ACC	AAG	1891
Thr	Thr	Asn	Gln	Ser	Ile	Ile	Ile	Gly	Gly	Leu	Phe	Pro	Gly	Thr	Lys	
		500				505					510					
TAT	TGC	TTT	GAA	ATA	GTT	CCA	AAA	GGA	CCA	AAT	GGG	ACT	GAA	GGG	GCA	1939
Tyr	Cys	Phe	Glu	Ile	Val	Pro	Lys	Gly	Pro	Asn	Gly	Thr	Glu	Gly	Ala	
515					520					525					530	
TCT	CGG	ACA	GTT	TGC	AAT	AGA	ACT	GTT	CCC	AGT	GCA	GTG	TTT	GAC	ATC	1987
Ser	Arg	Thr	Val	Cys	Asn	Arg	Thr	Val	Pro	Ser	Ala	Val	Phe	Asp	Ile	
				535					540					545		
CAC	GTG	GTC	TAC	GTC	ACC	ACC	ACG	GAG	ATG	TGG	CTG	GAC	TGG	AAG	AGC	2035
His	Val	Val	Tyr	Val	Thr	Thr	Thr	Glu	Met	Trp	Leu	Asp	Trp	Lys	Ser	
			550					555					560			
CCT	GAC	GGT	GCT	TCC	GAG	TAT	GTC	TAC	CAT	TTA	GTC	ATA	GAG	TCC	AAG	2083
Pro	Asp	Gly	Ala	Ser	Glu	Tyr	Val	Tyr	His	Leu	Val	Ile	Glu	Ser	Lys	
		565					570					575				
CAT	GGC	TCT	AAC	CAC	ACA	AGC	ACG	TAT	GAC	AAA	GCG	ATT	ACT	CTC	CAG	2131
His	Gly	Ser	Asn	His	Thr		Thr	Tyr	Asp	Lys	Ala	Ile	Thr	Leu	Gln	
	580					585					590					
GGC	CTG	ATT	CCG	GGC	ACC	TTA	TAT	AAC	ATC	ACC	ATC	TCT	CCA	GAA	GTG	2179
Gly	Leu	Ile	Pro	Gly	Thr	Leu	Tyr	Asn	Ile		Ile	Ser	Pro	Glu	Val	
595					600					605					610	
GAC	CAC	GTC	TGG	GGG	GAC	CCC	AAC	TCC	ACT	GCA	CAG	TAC	ACA	CGG	CCC	2227
Asp	His	Val	Trp	Gly	Asp	Pro	Asn	Ser	Thr	Ala	Gln	Tyr	Thr	Arg	Pro	
				615					620					625		
AGC	AAT	GTG	TCC	AAC	ATT	GAT	GTA	AGT	ACC	AAC	ACC	ACA	GCA	GCA	ACT	2275
Ser	Asn	Val	Ser	Asn	Ile	Asp	Val	Ser	Thr	Asn	Thr	Thr	Ala	Ala	Thr	
			630					635					640			
TTA	AGT	TGG	CAG	AAC	TTT	GAT	GAC	GCC	TCT	CCC	ACG	TAC	TCC	TAC	TGC	2323
Leu	Ser	Trp	Gln	Asn	Phe	Asp	Asp	Ala	Ser	Pro	Thr	Tyr	Ser	Tyr	Cys	
		645					650					655				
CTT	CTT	ATT	GAG	AAG	GCT	GGA	AAT	TCC	AGC	AAC	GCA	ACA	CAA	GTA	GTC	2371
Leu	Leu	Ile	Glu	Lys	Ala	Gly	Asn	Ser	Ser	Asn	Ala	Thr	Gln	Val	Val	
		660				665					670					
ACG	GAC	ATT	GGA	ATT	ACT	GAC	GCT	ACA	GTC	ACT	GAA	TTA	ATA	CCT	GGC	2419
Thr	Asp	Ile	Gly	Ile	Thr	Asp	Ala	Thr	Val	Thr	Glu	Leu	Ile	Pro	Gly	
					680					685					690	
TCA	TCA	TAC	ACA	GTG	GAG	CTC	TTT	GCA	CAA	GTA	GGG	GAT	GGG	ATC	AAG	2467
Ser	Ser	Tyr	Thr	Val	Glu	Leu	Phe	Ala	Gln	Val	Gly	Asp	Gly	Ile	Lys	

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695							700							705							
TCA Ser	CTG Leu	GAA Glu	CCT Pro 710	GGC Gly	CGG Arg	AAG Lys	TCA Ser	TTC Phe 715	TGT Cys	ACA Thr	GAT Asp	CCT Pro	GCG Ala 720	TCC Ser	ATG Met	2515					
GCC Ala	TCC Ser	TTC Phe 725	GAC Asp	TGC Cys	GAA Glu	GTG Val	GTC Val 730	CCC Pro	AAA Lys	GAG Glu	CCA Pro	GCC Ala 735	CTG Leu	GTT Val	CTC Leu	2563					
AAA Lys	TGG Trp 740	ACC Thr	TGC Cys	CCT Pro	CCT Pro	GGC Gly 745	GCC Ala	AAT Asn	GCA Ala	GGC Gly 750	TTT Phe	GAG Glu	CTG Leu	GAG Glu	GTC Val	2611					
AGC Ser 755	AGT Ser	GGA Gly	GCC Ala	TGG Trp	AAC Asn 760	AAT Asn	GCG Ala	ACC Thr	CAC His 765	CTG Leu	GAG Glu	AGC Ser	TGC Cys	TCC Ser	TCT Ser 770	2659					
GAG Glu	AAT Asn	GGC Gly	ACT Thr	GAG Glu 775	TAT Tyr	AGA Arg	ACG Thr	GAA Glu	GTC Val 780	ACG Thr	TAT Tyr	TTG Leu	AAT Asn	TTT Phe 785	TCT Ser	2707					
ACC Thr	TCG Ser	TAC Tyr	AAC Asn 790	ATC Ile	AGC Ser	ATC Ile	ACC Thr	ACT Thr 795	GTG Val	TCC Ser	TGT Cys	GGA Gly 800	AAG Lys	ATG Met	GCA Ala	2755					
GCC Ala	CCC Pro	ACC Thr 805	CGG Arg	AAC Asn	ACC Thr	TGC Cys	ACT Thr 810	ACT Thr	GGC Gly	ATC Ile	ACA Thr	GAT Asp 815	CCC Pro	CCT Pro	CCT Pro	2803					
CCA Pro	GAT Asp 820	GGA Gly	TCC Ser	CCT Pro	AAT Asn	ATT Ile 825	ACA Thr	TCT Ser	GTC Val	AGT Ser	CAC His 830	AAT Asn	TCA Ser	GTA Val	AAG Lys	2851					
GTC Val 835	AAG Lys	TTC Phe	AGT Ser	GGA Gly	TTT Phe 840	GAA Glu	GCC Ala	AGC Ser	CAC His	GGA Gly 845	CCC Pro	ATC Ile	AAA Lys	GCC Ala	TAT Tyr 850	2899					
GCT Ala	GTC Val	ATT Ile	CTC Leu	ACC Thr 855	ACC Thr	GGG Gly	GAA Glu	GCT Ala	GGT Gly 860	CAC His	CCT Pro	TCT Ser	GCA Ala	GAT Asp 865	GTC Val	2947					
CTG Leu	AAA Lys	TAC Tyr	ACG Thr 870	TAT Tyr	GAC Asp	GAT Asp	TTC Phe	AAA Lys 875	AAG Lys	GGA Gly	GCC Ala	TCA Ser	GAT Asp 880	ACT Thr	TAT Tyr	2995					
GTG Val	ACA Thr	TAC Tyr 885	CTC Leu	ATA Ile	AGA Arg	ACA Thr	GAA Glu 890	GAA Glu	AAG Lys	GGA Gly	CGT Arg	TCT Ser	CAG Gln	AGC Ser	TTG Leu	3043					
TCT Ser	GAA Glu 900	GTT Val	TTG Leu	AAA Lys	TAT Tyr	GAA Glu 905	ATT Ile	GAC Asp	GTT Val	GGG Gly 910	AAT Asn	GAG Glu	TCA Ser	ACC Thr	ACA Thr	3091					
CTT Leu 915	GGT Gly	TAT Tyr	TAC Tyr	AAT Asn	GGG Gly 920	AAG Lys	CTG Leu	GAA Glu	CCT Pro	CTG Leu 925	GGC Gly	TCC Ser	TAC Tyr	CGG Arg	GCT Ala 930	3139					
TGT Cys	GTG Val	GCT Ala	GGC Gly	TTC Phe 935	ACC Thr	AAC Asn	ATT Ile	ACC Thr	TTC Phe 940	CAC His	CCT Pro	CAA Gln	AAC Asn	AAG Lys 945	GGG Gly	3187					

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CTC ATT GAT GGG GCT GAG AGC TAT GTG TCC TTC AGT CGC TAC TCA GAT Leu Ile Asp Gly Ala Glu Ser Tyr Val Ser Phe Ser Arg Tyr Ser Asp 950 955 960	3235
GCT GTT TCC TTG CCC CAG GAT CCA GGT GTC ATC TGT GGA GCG GTT TTT Ala Val Ser Leu Pro Gln Asp Pro Gly Val Ile Cys Gly Ala Val Phe 965 970 975	3283
GGC TGT ATC TTT GGT GCC CTG GTT ATT GTG ACT GTG GGA GGC TTC ATC Gly Cys Ile Phe Gly Ala Leu Val Ile Val Thr Val Gly Gly Phe Ile 980 985 990	3331
TTC TGG AGA AAG AAG AGG AAA GAT GCA AAG AAT AAT GAA GTG TCC TTT Phe Trp Arg Lys Lys Arg Lys Asp Ala Lys Asn Asn Glu Val Ser Phe 995 1000 1005 1010	3379
TCT CAA ATT AAA CCT AAA AAA TCT AAG TTA ATC AGA GTG GAG AAT TTT Ser Gln Ile Lys Pro Lys Lys Ser Lys Leu Ile Arg Val Glu Asn Phe 1015 1020 1025	3427
GAG GCC TAC TTC AAG AAG CAG CAA GCT GAC TCC AAC TGT GGG TTC GCA Glu Ala Tyr Phe Lys Lys Gln Gln Ala Asp Ser Asn Cys Gly Phe Ala 1030 1035 1040	3475
GAG GAA TAC GAA GAT CTG AAG CTT GTT GGA ATT AGT CAA CCT AAA TAT Glu Glu Tyr Glu Asp Leu Lys Leu Val Gly Ile Ser Gln Pro Lys Tyr 1045 1050 1055	3523
GCA GCA GAA CTG GCT GAG AAT AGA GGA AAG AAT CGC TAT AAT AAT GTT Ala Ala Glu Leu Ala Glu Asn Arg Gly Lys Asn Arg Tyr Asn Asn Val 1060 1065 1070	3571
CTG CCC TAT GAT ATT TCC CGT GTC AAA CTT TCG GTC CAG ACC CAT TCA Leu Pro Tyr Asp Ile Ser Arg Val Lys Leu Ser Val Gln Thr His Ser 1075 1080 1085 1090	3619
ACG GAT GAC TAC ATC AAT GCC AAC TAC ATG CCT GGC TAC CAC TCC AAG Thr Asp Asp Tyr Ile Asn Ala Asn Tyr Met Pro Gly Tyr His Ser Lys 1095 1100 1105	3667
AAA GAT TTT ATT GCC ACA CAA GGA CCT TTA CCG AAC ACT TTG AAA GAT Lys Asp Phe Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr Leu Lys Asp 1110 1115 1120	3715
TTT TGG CGT ATG GTT TGG GAG AAA AAT GTA TAT GCC ATC ATT ATG TTG Phe Trp Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile Ile Met Leu 1125 1130 1135	3763
ACT AAA TGT GTT GAA CAG GGA AGA ACC AAA TGT GAG GAG TAT TGG CCC Thr Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu Glu Tyr Trp Pro 1140 1145 1150	3811
TCC AAG CAG GCT CAG GAC TAT GGA GAC ATA ACT GTG GCA ATG ACA TCA Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala Met Thr Ser 1155 1160 1165 1170	3859
GAA ATT GTT CTT CCG GAA TGG ACC ATC AGA GAT TTC ACA GTG AAA AAT Glu Ile Val Leu Pro Glu Trp Thr Ile Arg Asp Phe Thr Val Lys Asn 1175 1180 1185	3907
ATC CAG ACA AGT GAG AGT CAC CCT CTG AGA CAG TTC CAT TTC ACC TCC	3955

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Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe His Phe Thr Ser	
1190 1195 1200	
TGG CCA GAC CAC GGT GTT CCC GAC ACC ACT GAC CTG CTC ATC AAC TTC	4003
Trp Pro Asp His Gly Val Pro Asp Thr Thr Asp Leu Leu Ile Asn Phe	
1205 1210 1215	
CGG TAC CTC GTT CGT GAC TAC ATG AAG CAG AGT CCT CCC GAA TCG CCG	4051
Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln Ser Pro Pro Glu Ser Pro	
1220 1225 1230	
ATT CTG GTG CAT TGC AGT GCT GGG GTC GGA AGG ACG GGC ACT TTC ATT	4099
Ile Leu Val His Cys Ser Ala Gly Val Gly Arg Thr Gly Thr Phe Ile	
1235 1240 1245 1250	
GCC ATT GAT CGT CTC ATC TAC CAG ATA GAG AAT GAG AAC ACC GTG GAT	4147
Ala Ile Asp Arg Leu Ile Tyr Gln Ile Glu Asn Glu Asn Thr Val Asp	
1255 1260 1265	
GTG TAT GGG ATT GTG TAT GAC CTT CGA ATG CAT AGG CCT TTA ATG GTG	4195
Val Tyr Gly Ile Val Tyr Asp Leu Arg Met His Arg Pro Leu Met Val	
1270 1275 1280	
CAG ACA GAG GAC CAG TAT GTT TTC CTC AAT CAG TGT GTT TTG GAT ATT	4243
Gln Thr Glu Asp Gln Tyr Val Phe Leu Asn Gln Cys Val Leu Asp Ile	
1285 1290 1295	
GTC AGA TCC CAG AAA GAC TCA AAA GTA GAT CTT ATC TAC CAG AAC ACA	4291
Val Arg Ser Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr	
1300 1305 1310	
ACT GCA ATG ACA ATC TAT GAA AAC CTT GCG CCC GTG ACC ACA TTT GGA	4339
Thr Ala Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly	
1315 1320 1325 1330	
AAG ACC AAT GGT TAC ATC GCC TAATTCCAAA GGAATAACCT TTCT	4384
Lys Thr Asn Gly Tyr Ile Ala	
1335	
GGAGTGAACC AGACCGTCGC ACCCACAGCG AAGGCACATG CCCCAGATGTC GACATGTTTT	4444
TATATGTCTA ATATCTTAAT TCTTTGTTCT GTTTTGTGAG AACTAATTTT GAGGGCATGA	4504
AGCTGCATAT GATAGATGAC AAATTGGGGC TGTCGGGGGC TGTGGATGGG TGGGGAGCAA	4564
ATCATCTGCA TTCCTGATGA CCAATGGGAT GAGGTCACCT TTTTTTTTTT CCCCCTTGAG	4624
GATTGCGGAA AACCAGGAAA AGGGATCTAT GATTTTTTTT TCCAAAACAA TTTCTTTTTT	4684
AAAAAGACTA TTTTATATGA TTCACATGCT AAAGCCAGGA TTGTGTTGGG TTGAATATAT	4744
TTTAAGTATC AGAGGTCTAT TTTTACCTAC TGTGTCTTGG AATCTAGCCG ATGGAAAATA	4804
CCTAATTGTG GATGATGATT GCGCAGGGAG GGGTACGTGG CACCTCTTCC GAATGGGTTT	4864
TCTATTTGAA CATGTGCCTT TTCTGAATTA TGCTTCCACA GGCAAACTC AGTAGAGATC	4924
TATATTTTGG TACTGAATCT CATAATTGGA ATATACGGAA TATTTAAACA GTAGCTTAGC	4984
ATCAGAGGTT TGCTTCCTCA GTAACATTTT TGTTCCTCATT TGATCAGGGG AGGCCTCTTT	5044

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GCGCCGGCCC CGCTTCCCCT GCGCCCGTGT GATTGTGCT CCATTTTTC TTCCCTTTTC 5104
 CCTCCCAGTT TTC 5117

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1337 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Pro Ala Ala Arg Glu Ala Arg Leu Pro Pro Arg Ser Pro Gly
 1 5 10 15
 Leu Arg Trp Ala Leu Pro Leu Leu Leu Leu Leu Arg Leu Gly Gln
 20 25 30
 Ile Leu Cys Ala Gly Gly Thr Pro Ser Pro Ile Pro Asp Pro Ser Val
 35 40 45
 Ala Thr Val Ala Thr Gly Glu Asn Gly Ile Thr Gln Ile Ser Ser Thr
 50 55 60
 Ala Glu Ser Phe His Lys Gln Asn Gly Thr Gly Thr Pro Gln Val Glu
 65 70 75 80
 Thr Asn Thr Ser Glu Asp Gly Glu Ser Ser Gly Ala Asn Asp Ser Leu
 85 90 95
 Arg Thr Pro Glu Gln Gly Ser Asn Gly Thr Asp Gly Ala Ser Gln Lys
 100 105 110
 Thr Pro Ser Ser Thr Gly Pro Ser Pro Val Phe Asp Ile Lys Ala Val
 115 120 125
 Ser Ile Ser Pro Thr Asn Val Ile Leu Thr Trp Lys Ser Asn Asp Thr
 130 135 140
 Ala Ala Ser Glu Tyr Lys Tyr Val Val Lys His Lys Met Glu Asn Glu
 145 150 155 160
 Lys Thr Ile Thr Val Val His Gln Pro Trp Cys Asn Ile Thr Gly Leu
 165 170 175
 Arg Pro Ala Thr Ser Tyr Val Phe Ser Ile Thr Pro Gly Ile Gly Asn
 180 185 190
 Glu Thr Trp Gly Asp Pro Arg Val Ile Lys Val Ile Thr Glu Pro Ile
 195 200 205
 Pro Val Ser Asp Leu Arg Val Ala His Gly Cys Glu Glu Gly Cys Ser
 210 215 220
 Leu Ser Trp Ser Asn Gly Asn Gly Thr Ala Ser Cys Arg Val Leu Leu
 225 230 235 240

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Glu 245	Ser 245	His 245	Glu 245	Glu 245	Leu 245	Thr 245	Gln 245	Asp 245	Ser 245	Arg 245	Leu 245	Gln 245			
Val 260	Asn 260	Ile 260	Ser 260	Asp 260	Leu 260	Lys 260	Pro 260	Gly 260	Val 260	Gln 260	Tyr 260	Asn 260	Ile 260	Asn 260	Pro 260
Tyr 275	Leu 275	Leu 275	Gln 275	Ser 275	Asn 275	Lys 275	Thr 275	Lys 275	Gly 275	Asp 275	Pro 275	Leu 275	Ala 275	Gln 275	Lys 275
Val 290	Ala 290	Trp 290	Met 290	Pro 290	Ala 290	Ile 290	Gln 290	Arg 290	Glu 290	Ala 290	Gly 290	Gln 290	Gly 290	Ala 290	Pro 290
Pro 305	Pro 305	Leu 305	Cys 305	Met 305	Met 305	Ser 305	Pro 305	Phe 305	Val 305	Gly 305	Pro 305	Val 305	Asp 305	Pro 305	Ser 305
Ser 320	Gly 320	Gln 320	Gln 320	Ser 320	Arg 320	Asp 320	Thr 320	Glu 320	Val 320	Leu 320	Leu 320	Val 320	Gly 320	Leu 320	Glu 320
Pro 340	Gly 340	Thr 340	Arg 340	Tyr 340	Asn 340	Ala 340	Thr 340	Val 340	Tyr 340	Ser 340	Gln 340	Ala 340	Ala 340	Asn 340	Gly 340
Thr 355	Glu 355	Gly 355	Gln 355	Pro 355	Gln 355	Ala 355	Ile 355	Glu 355	Phe 355	Arg 355	Thr 355	Asn 355	Ala 355	Ile 355	Gln 355
Val 370	Phe 370	Asp 370	Val 370	Thr 370	Ala 370	Val 370	Asn 370	Ile 370	Ser 370	Ala 370	Thr 370	Ser 370	Leu 370	Thr 370	Leu 370
Ile 385	Trp 385	Lys 385	Val 385	Ser 385	Asp 385	Asn 385	Glu 385	Ser 385	Ser 385	Ser 385	Asn 385	Tyr 385	Thr 385	Tyr 385	Lys 385
Ile 400	His 400	Val 400	Ala 400	Gly 400	Glu 400	Thr 400	Asp 400	Ser 400	Ser 400	Asn 400	Leu 400	Asn 400	Val 400	Ser 400	Glu 400
Pro 420	Arg 420	Ala 420	Val 420	Ile 420	Pro 420	Gly 420	Leu 420	Arg 420	Ser 420	Ser 420	Thr 420	Phe 420	Tyr 420	Asn 420	Ile 420
Thr 435	Val 435	Cys 435	Pro 435	Val 435	Leu 435	Gly 435	Asp 435	Ile 435	Glu 435	Gly 435	Thr 435	Pro 435	Gly 435	Phe 435	Leu 435
Gln 450	Val 450	His 450	Thr 450	Pro 450	Pro 450	Val 450	Pro 450	Val 450	Ser 450	Asp 450	Phe 450	Arg 450	Val 450	Thr 450	Val 450
Val 465	Ser 465	Thr 465	Thr 465	Glu 465	Ile 465	Gly 465	Leu 465	Ala 465	Trp 465	Ser 465	Ser 465	His 465	Asp 465	Ala 465	Glu 465
Ser 480	Phe 480	Gln 480	Met 480	His 480	Ile 480	Thr 480	Gln 480	Glu 480	Gly 480	Ala 480	Gly 480	Asn 480	Ser 480	Arg 480	Val 480
Glu 500	Ile 500	Thr 500	Thr 500	Asn 500	Gln 500	Ser 500	Ile 500	Ile 500	Ile 500	Gly 500	Gly 500	Leu 500	Phe 500	Pro 500	Gly 500
Thr 515	Lys 515	Tyr 515	Cys 515	Phe 515	Glu 515	Ile 515	Val 515	Pro 515	Lys 515	Gly 515	Pro 515	Asn 515	Gly 515	Thr 515	Glu 515
Gly 530	Ala 530	Ser 530	Arg 530	Thr 530	Val 530	Cys 530	Asn 530	Arg 530	Thr 530	Val 530	Pro 530	Ser 530	Ala 530	Val 530	Phe 530
Asp 545	Ile 545	His 545	Val 545	Val 545	Tyr 545	Val 545	Thr 545	Thr 545	Thr 545	Glu 545	Met 545	Trp 545	Leu 545	Asp 545	Trp 545
Lys 560	Ser 560	Pro 560	Asp 560	Gly 560	Ala 560	Ser 560	Glu 560	Tyr 560	Val 560	Tyr 560	His 560	Leu 560	Val 560	Ile 560	Glu 560

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565					570					575					
Ser	Lys	His	Gly	Ser	Asn	His	Thr	Ser	Thr	Tyr	Asp	Lys	Ala	Ile	Thr
			580					585					590		
Leu	Gln	Gly	Leu	Ile	Pro	Gly	Thr	Leu	Tyr	Asn	Ile	Thr	Ile	Ser	Pro
		595					600					605			
Glu	Val	Asp	His	Val	Trp	Gly	Asp	Pro	Asn	Ser	Thr	Ala	Gln	Tyr	Thr
	610					615					620				
Arg	Pro	Ser	Asn	Val	Ser	Asn	Ile	Asp	Val	Ser	Thr	Asn	Thr	Thr	Ala
625						630					635				640
Ala	Thr	Leu	Ser	Trp	Gln	Asn	Phe	Asp	Asp	Ala	Ser	Pro	Thr	Tyr	Ser
				645					650					655	
Tyr	Cys	Leu	Leu	Ile	Glu	Lys	Ala	Gly	Asn	Ser	Ser	Asn	Ala	Thr	Gln
			660					665					670		
Val	Val	Thr	Asp	Ile	Gly	Ile	Thr	Asp	Ala	Thr	Val	Thr	Glu	Leu	Ile
		675					680					685			
Pro	Gly	Ser	Ser	Tyr	Thr	Val	Glu	Leu	Phe	Ala	Gln	Val	Gly	Asp	Gly
	690					695					700				
Ile	Lys	Ser	Leu	Glu	Pro	Gly	Arg	Lys	Ser	Phe	Cys	Thr	Asp	Pro	Ala
705						710					715				720
Ser	Met	Ala	Ser	Phe	Asp	Cys	Glu	Val	Val	Pro	Lys	Glu	Pro	Ala	Leu
				725					730					735	
Val	Leu	Lys	Trp	Thr	Cys	Pro	Pro	Gly	Ala	Asn	Ala	Gly	Phe	Glu	Leu
			740					745					750		
Glu	Val	Ser	Ser	Gly	Ala	Trp	Asn	Asn	Ala	Thr	His	Leu	Glu	Ser	Cys
		755					760					765			
Ser	Ser	Glu	Asn	Gly	Thr	Glu	Tyr	Arg	Thr	Glu	Val	Thr	Tyr	Leu	Asn
		770				775					780				
Phe	Ser	Thr	Ser	Tyr	Asn	Ile	Ser	Ile	Thr	Thr	Val	Ser	Cys	Gly	Lys
785						790					795				800
Met	Ala	Ala	Pro	Thr	Arg	Asn	Thr	Cys	Thr	Thr	Gly	Ile	Thr	Asp	Pro
				805					810					815	
Pro	Pro	Pro	Asp	Gly	Ser	Pro	Asn	Ile	Thr	Ser	Val	Ser	His	Asn	Ser
			820					825					830		
Val	Lys	Val	Lys	Phe	Ser	Gly	Phe	Glu	Ala	Ser	His	Gly	Pro	Ile	Lys
		835					840					845			
Ala	Tyr	Ala	Val	Ile	Leu	Thr	Thr	Gly	Glu	Ala	Gly	His	Pro	Ser	Ala
	850					855					860				
Asp	Val	Leu	Lys	Tyr	Thr	Tyr	Asp	Asp	Phe	Lys	Lys	Gly	Ala	Ser	Asp
865						870					875				880
Thr	Tyr	Val	Thr	Tyr	Leu	Ile	Arg	Thr	Glu	Glu	Lys	Gly	Arg	Ser	Gln
				885					890					895	

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Ser Leu Ser Glu Val Leu Lys Tyr Glu Ile Asp Val Gly Asn Glu Ser
 900 905 910
 Thr Thr Leu Gly Tyr Tyr Asn Gly Lys Leu Glu Pro Leu Gly Ser Tyr
 915 920 925
 Arg Ala Cys Val Ala Gly Phe Thr Asn Ile Thr Phe His Pro Gln Asn
 930 935 940
 Lys Gly Leu Ile Asp Gly Ala Glu Ser Tyr Val Ser Phe Ser Arg Tyr
 945 950 955 960
 Ser Asp Ala Val Ser Leu Pro Gln Asp Pro Gly Val Ile Cys Gly Ala
 965 970 975
 Val Phe Gly Cys Ile Phe Gly Ala Leu Val Ile Val Thr Val Gly Gly
 980 985 990
 Phe Ile Phe Trp Arg Lys Lys Arg Lys Asp Ala Lys Asn Asn Glu Val
 995 1000 1005
 Ser Phe Ser Gln Ile Lys Pro Lys Lys Ser Lys Leu Ile Arg Val Glu
 1010 1015 1020
 Asn Phe Glu Ala Tyr Phe Lys Lys Gln Gln Ala Asp Ser Asn Cys Gly
 1025 1030 1035 1040
 Phe Ala Glu Glu Tyr Glu Asp Leu Lys Leu Val Gly Ile Ser Gln Pro
 1045 1050 1055
 Lys Tyr Ala Ala Glu Leu Ala Glu Asn Arg Gly Lys Asn Arg Tyr Asn
 1060 1065 1070
 Asn Val Leu Pro Tyr Asp Ile Ser Arg Val Lys Leu Ser Val Gln Thr
 1075 1080 1085
 His Ser Thr Asp Asp Tyr Ile Asn Ala Asn Tyr Met Pro Gly Tyr His
 1090 1095 1100
 Ser Lys Lys Asp Phe Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr Leu
 1105 1110 1115 1120
 Lys Asp Phe Trp Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile Ile
 1125 1130 1135
 Met Leu Thr Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu Glu Tyr
 1140 1145 1150
 Trp Pro Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala Met
 1155 1160 1165
 Thr Ser Glu Ile Val Leu Pro Glu Trp Thr Ile Arg Asp Phe Thr Val
 1170 1175 1180
 Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe His Phe
 1185 1190 1195 1200
 Thr Ser Trp Pro Asp His Gly Val Pro Asp Thr Thr Asp Leu Leu Ile
 1205 1210 1215
 Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln Ser Pro Pro Glu

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1220	1225	1230
Ser Pro Ile Leu Val His Cys Ser Ala Gly Val Gly Arg Thr Gly Thr		
1235	1240	1245
Phe Ile Ala Ile Asp Arg Leu Ile Tyr Gln Ile Glu Asn Glu Asn Thr		
1250	1255	1260
Val Asp Val Tyr Gly Ile Val Tyr Asp Leu Arg Met His Arg Pro Leu		
1265	1270	1275
Met Val Gln Thr Glu Asp Gln Tyr Val Phe Leu Asn Gln Cys Val Leu		
1285	1290	1295
Asp Ile Val Arg Ser Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr Gln		
1300	1305	1310
Asn Thr Thr Ala Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr Thr		
1315	1320	1325
Phe Gly Lys Thr Asn Gly Tyr Ile Ala		
1330	1335	

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Lys Cys Ala Gln Tyr Trp Pro
 1 5

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

His Cys Ser Ala Gly Ile Gly
 1 5

- 39 -

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AARTGYGCNC ARTAYTGCC

20

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE

- (D) OTHER INFORMATION: /note= "Base designated N at
postion 6 is Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCDATNCCNG CRCTRCARTG

20

- 40 -

WHAT IS CLAIMED IS:

1. A purified and isolated density enhanced protein tyrosine phosphatase polypeptide.
2. A receptor-like protein tyrosine phosphatase polypeptide according to claim 1.
3. A Type III receptor-like protein tyrosine phosphatase polypeptide according to claim 1.
4. The Type III receptor-like protein tyrosine phosphatase polypeptide according to claim 3 consisting essentially of the huDEP-1 amino acid sequence set out in SEQ ID NO: 2, or a variant thereof.
5. A polynucleotide encoding the protein tyrosine phosphatase polypeptide of claim 1.
6. The polynucleotide of claim 5 which is a DNA.
7. The DNA of claim 6 which is selected from the group consisting of genomic DNA, cDNA, partially chemically synthesized DNA, and wholly chemically synthesized DNA.
8. The DNA of claim 6 further comprising regulatory DNA sequences which direct transcription of the DNA.
9. A DNA expression construct comprising the DNA of claim 8.

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10. A host cell transformed or transfected with the DNA of claim 6.

11. A method for producing a density enhanced protein tyrosine phosphatase polypeptide comprising growing a host cell according to claim 10 in a suitable medium and isolating the phosphatase polypeptide from the host cell or the medium of its growth.

12. A purified and isolated polynucleotide encoding a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide.

13. The polynucleotide according to claim 12 which is a DNA.

14. The DNA of claim 13 which is selected from the group consisting of cDNA, genomic DNA, partially chemically synthesized DNA, and wholly chemically synthesized DNA.

15. The DNA of claim 13 comprising a huDEP-1 protein coding sequence as set forth in SEQ ID NO: 1, or a variant thereof.

16. The DNA of claim 13 further comprising regulatory DNA sequences which direct transcription of the DNA.

17. A purified and isolated polynucleotide selected from the group consisting of:

- a) the DNA sequence set out in SEQ ID NO: 1, and
- b) a DNA molecule which hybridizes under stringent conditions to the protein coding portion of the DNA of (a).

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18. A DNA encoding a huDEP-1 amino acid sequence set out in SEQ ID NO: 2, or a variant thereof.
19. A DNA expression construct comprising the DNA of claim 16.
20. A host cell transformed or transfected with the DNA of claim 13.
21. A method for producing a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide comprising the steps of growing the host cell according to claim 20 in a suitable medium and isolating the polypeptide from the host cell or the medium of its growth.
22. The method of claim 21 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase is huDEP-1, or a variant thereof.
23. A polypeptide or peptide capable of specifically binding to a density enhanced protein tyrosine phosphatase polypeptide.
24. A polypeptide or peptide capable of specifically binding to a density enhanced Type III' receptor-like protein tyrosine phosphatase polypeptide.
25. The polypeptide according to claim 24 which is an antibody.

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26. The antibody according to claim 25 which is a monoclonal antibody.

27. An anti-idiotypic antibody specific for the monoclonal antibody of claim 26.

28. A hybridoma cell line producing the antibody of claim 26 or 27.

29. The polypeptide or peptide according to claims 24, 25, 26, or 27 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide is huDEP-1, or a variant thereof.

30. A method for isolating a polynucleotide encoding a polypeptide that binds to a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide comprising the steps of:

a) transforming or transfecting host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA-binding domain and an activating domain;

b) expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide and either the DNA-binding domain or the activating domain of the transcription factor;

c) expressing in the host cells a library of second hybrid DNA sequences encoding second fusions of part or all of putative density enhanced Type III receptor-like protein tyrosine phosphatase binding polypeptides and either the activating domain or DNA binding domain of the transcription factor which is not incorporated in the first fusion;

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d) transforming or transfecting the host cells with a DNA construct comprising a protein tyrosine kinase gene;

e) detecting binding of density enhanced Type III receptor-like protein tyrosine phosphatase binding polypeptide(s) to the phosphatase polypeptide in the host cell by detecting the production of reporter gene product in the host cell(s); and

f) isolating the second hybrid DNA sequences encoding the phosphatase binding polypeptide from the host cell(s).

31. The method of claim 30 wherein the promoter is the beta-galactosidase promoter, the DNA-binding domain is the *lexA* DNA-binding domain, the activating domain is the GAL4 transactivation domain, the reporter gene is the *lacZ* gene and the host cells are yeast host cells.

32. The method according to claim 30 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase is huDEP-1, or a variant thereof.

33. The method according to claim 30 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase is a catalytically inactive variant of huDEP-1 capable of binding huDEP-1 substrate.

34. A method for detecting proteins which bind to a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide isoform comprising the steps of:

a) transforming or transfecting host cells with a hybrid DNA sequence encoding a fusion between a putative phosphatase binding protein and a ligand capable of high affinity binding to a specific counterreceptor;

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- b) expressing the hybrid DNA sequence in the host cells under appropriate conditions;
- c) immobilizing fusion protein from the host cells by exposing the fusion protein to the specific counterreceptor in immobilized form;
- d) contacting the density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide with the immobilized fusion protein; and
- e) detecting the phosphatase polypeptide bound to the fusion protein using a reagent specific for the phosphatase polypeptide.

35. The method of claim 34 wherein the ligand is glutathione-S-transferase and the counterreceptor is glutathione.

36. The method of claim 34 wherein the ligand is hemagglutinin and the counterreceptor is a hemagglutinin-specific antibody.

37. The method of claim 34 wherein the ligand is polyhistidine and the counterreceptor is nickel.

38. The method of claim 34 wherein the ligand is maltose-binding protein and the counterreceptor is amylose.

39. The method of claim 33 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide is huDEP-1, or a variant thereof.

1 / 3

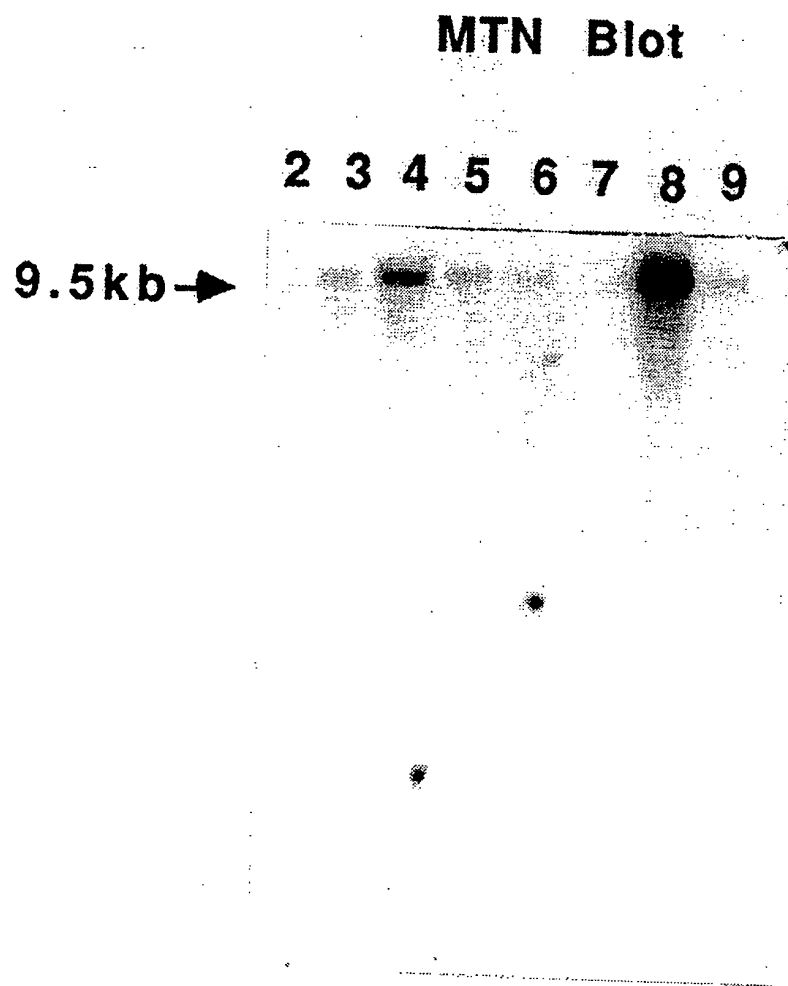


FIG. 1A

2 / 3

MTN Blot II

2 3 4 5 6 7 8 9

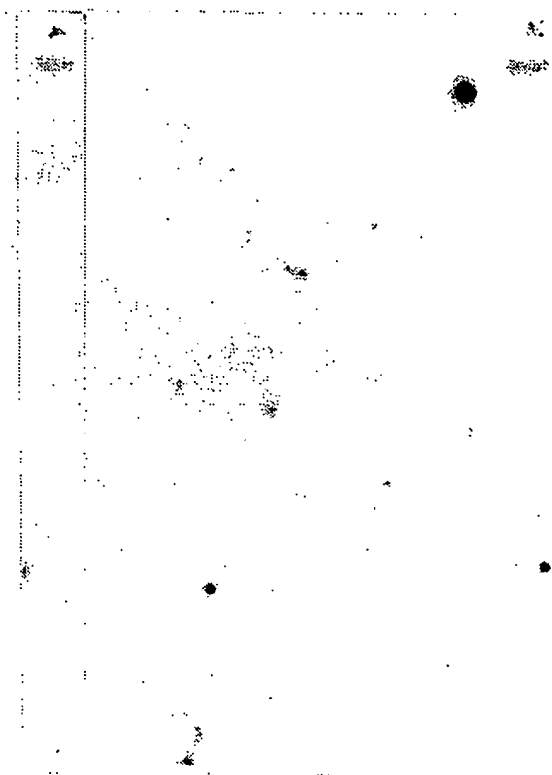


FIG. 1B

3 / 3

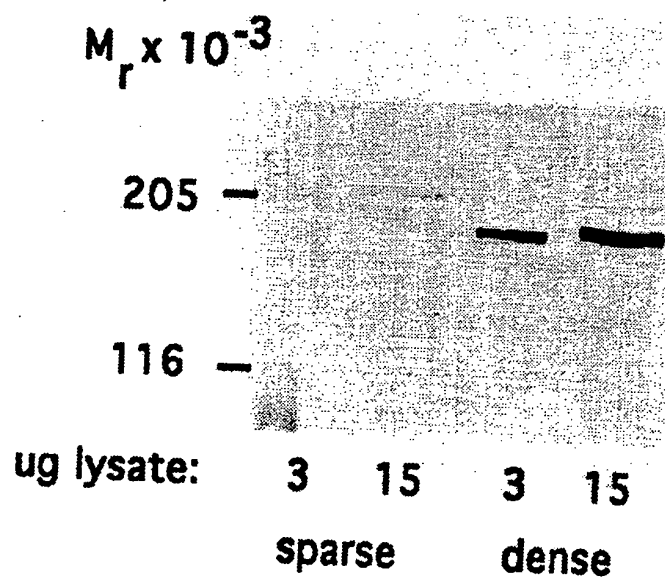


FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/05512

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/55 C12N9/16 C12N5/10 C07K16/40 C07K16/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 88, August 1991 WASHINGTON US, pages 6996-7000, PALLAN AND TONG 'Elevation of membrane tyrosine phosphatase activity in density-dependent growth-arrested fibroblasts' cited in the application see the whole document</p> <p style="text-align: center;">--- -/-</p>	1,23

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *A* document member of the same patent family

Date of the actual completion of the international search

26 July 1995

Date of mailing of the international search report

16.08.95

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/05512

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY , vol. 269, no. 3, 21 January 1994 MD US, pages 2075-2081, MATOZAKI ET AL. 'Molecular cloning of a human transmembrane-type proteon tyrosine phosphatase and its expression in gastrointestinal cancers' cited in the application see the whole document ---	1-39
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, October 1994 WASHINGTON US, pages 9680-9684, OSTMAN ET AL. 'Expression of DEP-1, a receptor-like protein-tyrosine-phosphatase, is enhanced with increasing cell density' see the whole document ---	1-39
X,P	BLOOD, vol. 84, no. 12 , 15 December 1994 pages 4186-4194, HONDA ET AL. 'Molecular cloning, characterization, and chromosomal location of a novel protein-tyrosine phosphatase, HPTPeta' see figure 3 which has over 99% identity with SEQ ID NO:1 -----	1-39